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Response of multiple seeded cocklebur and other cocklebur types to herbicide treatment

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Abstract: Multiple seeded cocklebur has been found in the last decade in Texas, and described as a biotype of *Xanthium strumarium* L with up to 25 seeds per bur instead of the usual two. The multiple seeded bur typically produces up to nine seedlings, causing concern that it may be harder to control than normal seeded common cocklebur. The efficacies of a series of fungal and conventional commercial herbicides have been compared in the greenhouse on seedlings of multiple seeded cocklebur from Texas (MSC-TX) and normal common cockleburs from Texas (NCC-TX), Arkansas (NCC-AR), Illinois (NCC-ILL) and two from Mississippi (NCC-MS#1, NCC-MS#2). Three measures of herbicidal activity (reductions in plant height and dry weight, and mortality) were used. The fungal herbicide *Alternaria helianthi* (Hansf) Tubaki & Nishihara at 1 x 10^5 conidia ml^{-1} + 2 g $liter^{-1}$ Silwet L-77 with an 8-h dew period was an effective herbicide with all biotypes, as were the commercial chemical herbicides chlorimuron (14.8 g ha^{-1}), imazaquin (29.6 g ha^{-1}), sodium hydrogen methylarsonate (MSMA; 279.1 g ha^{-1}) and imazethapyr (39.5 g ha^{-1}). The membrane-disrupting organic arsenical MSMA was effective with all biotypes, whereas commercial chemical herbicides which act by inhibiting branched-chain amino acid synthesis (chlorimuron, imazaquin and imazethapyr) were less effective against normal seeded common cocklebur biotypes with short stature. These studies showed that multiple seeded cocklebur was at least as susceptible to the biological agent *A helianthi* and to the conventional commercial herbicides studied as were normal seeded cockleburs, suggesting that existing methods should be adequate to control this novel biotype.

Keywords: common cocklebur; *Xanthium strumarium*; cocklebur biotype; multiple seeded cocklebur; *Alternaria helianthi*; biological control; chemical control; formulation

1 INTRODUCTION

Common cocklebur (*Xanthium strumarium* L) is an economically significant annual weed in many crops including cotton, corn and soybean.[1–4] Species of the genus *Xanthium* (family Compositae) are troublesome weeds throughout most of the world.[5] Common cocklebur can also be found on beaches, along watercourses and in recreational areas.[6] Common cocklebur has many varieties including those resistant to conventional herbicides such as MSMA (sodium hydrogen methylarsenate),[7–10] imazaquin,[11] chlorimuron,[12,13] (Ronald E Talbert, personal communication) and imazethapyr.[12,14] The genus *Xanthium* contains varieties that vary in their growth and development.[15–20] A unique biotype, called multiple seeded cocklebur (MSC), was discovered in Bell County, TX, in 1994.[21] This biotype has up to 25 seeds per bur instead of the usual two, and it usually produces up to nine seedlings per bur. MSC burs are large, round and flattened on one end.[21]

Alternaria species are important pathogens of a wide variety of weed and crop species.[22–24] *Alternaria helianthi* (Hansf) Tubaki & Nishihara has now been well documented as an effective pathogen to control common cocklebur.[22,23,25,26] However, the efficacy of conventional herbicides and *A helianthi* has not been previously evaluated on MSC. Therefore, in the present study, we compared the response of MSC with that of normal common cocklebur (NCC) varieties to the mycoherbicide agent *A helianthi*[25] and to several conventional herbicides which are known to control the latter[27–30] in various geographical areas.

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2 MATERIALS AND METHODS

2.1 Cocklebur

Seeds of MSC and normal common cocklebur from Texas (NCC-TX) were collected from plants growing in experimental plots at the Southern Weed Science Research Nursery, Stoneville, Mississippi. The original sources of seeds of both biotypes were as described elsewhere.[21] Normal common cocklebur, Mississippi #1 and Mississippi #2, with seeds (NCC-MS #1 and NCC-MS #2, respectively), were collected from two different local areas near Stoneville, MS. Chlorimuron-sensitive common cocklebur seeds (NCC-AR) were a generous gift from Dr RE Talbert, University of Arkansas, Agronomy, Fayetteville, AR. Imazethapyr-sensitive common cocklebur seeds (NCC-IL) were collected by Wax and Hine from experimental plots in the field at DSAC, IL. All burs except MSC burs were divided by a jeweler's saw between the two beaks, lengthwise, while attempting to avoid any damage to the seeds, as described previously.[21] Comparisons of all biotypes were studied in the greenhouse. Comparisons included the responses of all biotypes to various control agents, which included the biocontrol agent *A helianthi* as well as chemical herbicides.

2.2 Mycoherbicide

Alternaria helianthi, a mycoherbicide for MSC-TX and NCC biotypes of cocklebur, was originally obtained from C Block, USDA-ARS, Ames, IA. Conidia used in the present experiments were obtained from cultures reisolated from infected cocklebur plants grown in the field (microplots, each 2×2 m) at the Southern Weed Science Research Unit, Stoneville, MS. *Alternaria helianthi* was included in the present study as a positive control using the optimal adjuvant and dew period as were determined previously.[25] The adjuvant was Silwet L-77 (SW), an organosilicone surfactant (polyalkyleneoxide-modified heptamethyltrisiloxane) obtained from Union Carbide, Tarrytown, NY, USA. The fungal inoculum was prepared as described previously.[31] Suspensions of *A helianthi* conidia (1×10^5 conidia ml^{-1} in 2 g liter^{-1} aqueous SW) prepared as described previously[22] were sprayed to run-off on cocklebur seedlings using a Spra-Tool (Crown Industrial Products Co, Hebron, IL, USA). Treated and control plants were subjected to an 8-h dew period prior to being transferred to the greenhouse. Cross-contamination from infected to control plants did not occur.

2.3 Chemical herbicides

In this study four chemical herbicides, chlorimuron, imazaquin, imazethapyr and MSMA, were chosen that are widely used to control common cocklebur in various geographical areas.[27–30,32,33] They were used as commercial formulations: chlorimuron 250 g kg^{-1} DG (CLASSIC®, DuPont), imazaquin 700 g kg^{-1} DG (Scepter®, American Cyanamid), imazethapyr 700 g kg^{-1} DG (Pursuit®, American Cyanamid) and MSMA 510 g liter^{-1} SL (Luxembourg & ISK Biosciences). Herbicide dispersions were prepared by diluting the formulation in water, and then sprayed on the top of plants, using an indoor spray table with an air pressure spray system. Each herbicide was applied at 187 liter ha^{-1} and 138 kpa with Teejet 8002E nozzles in a Sprayer Chamber (Allen Machine Works, Midland, Mich, USA). Five different rates of each herbicide were initially used: 0.25X, 0.5X, X, 2X, and 4X (for chlorimuron X = 29.6 g ha^{-1}, imazaquin X = 113.1 g ha^{-1}, MSMA X = 279.1 g ha^{-1} and imazethapyr: X = 158.1 g ha^{-1}). These ranges covered the lowest concentration of each herbicide which killed all or most of the cocklebur seedlings.

2.4 Herbicide treatment

The plants for this test were prepared using procedures similar to those described in detail previously.[22] MSC-TX burs were soaked in running water for a week before planting in a potting mix of Jiffy Mix + soil (1 + 1 by weight; Jiffy Mix: Jiffy Products of America, Inc, Batavia, IL 60 510). Seeds of all NCC biotypes were incubated in water in Petri dishes for 24–72 h to remove the seed coat and to select the healthy germinated seeds. Germinated seeds of all NCC biotypes were transplanted into individual 10-cm pots in the same soil mixture, and grown in the greenhouse until the 6–8 leaf stage (14–21 days old). Four replicates with 10 plants each were used for each treatment. After treatment, plants were transferred to the greenhouse, where they were kept for 14 days. Plants were watered as needed and fertilized with N:P:K, 20:20:20. Plant height, dry weight and mortality were measured at the end of the experiment (14 days).

2.5 Statistical analysis

The experiment was conducted in a randomized complete block design with four replicates, each containing 10 plants (one plant per pot). Components of the experiment were repeated two or more times. Results were expressed as mean ± the standard deviation calculated using the statistical package in Microsoft Excel 97 software. Because of non-random distributions, it was not possible to carry out valid statistical analysis on the plant height or weight data by the parametric tests evaluated, or on mortality data by the non-parametric tests evaluated. Statistical analysis of both plant height and dry weight data by the non-parametric Kruskal–Wallis test indicated that for each treatment differences among biotypes were significant ($P < 0.05$), and for each biotype differences among treatments were significant ($P < 0.02$). The Exact Wilcoxon test was subsequently used to determine (i) which biotypes differed significantly (two-sided P-value <0.05) in dry weight and plant height from the multi-seeded cocklebur, MSC-TX; and (ii) which chemical treatments resulted in significantly (two-sided P-value <0.05) different dry weight and plant height measurements.

3 RESULTS AND DISCUSSION

The novel biotype, multiple seeded cocklebur from Texas (MSC-TX), was compared with five biotypes of normal seeded cocklebur for susceptibility to a series of conventional commercial chemical herbicides in the greenhouse. The mycoherbicide *Alternaria helianthi* was used as a positive control. Previous studies[23,25,26] had shown that the latter is an effective herbicide against cocklebur when sprayed at 1×10^5 conidia ml^{-1} in 2 g liter^{-1} aqueous Silwet L-77 followed by an 8-h dew period. Plants treated with 2 g liter^{-1} aqueous Silwet L-77 were used as negative controls. Herbicidal activity was measured by plant height reduction, reduction of biomass dry weight and mortality (Table 1). Plant height reduction was the most effective measure of herbicidal activity, identifying significant differences between treatments more often than dry weight or mortality. Symptoms of disease on treated plants started as soft necrotic lesions on leaf surfaces and stems, which became more severe and larger with time so as to cover the whole plant and resulting in mortality of plants within 1 week (Fig 1a and b).

Plants treated with *A helianthi* at 1×10^5 conidia ml^{-1} aqueous SW showed dramatic damage in all measurements of herbicidal activity (Table 1). The damage by *A helianthi* on MSC plants followed the same trend as on normal seeded biotypes, except that the effects of *A helianthi* were more dramatic on MSC plants (Table 1). *Alternaria helianthi* caused 100% mortality to 6- to 12-leaf-stage plants of biotypes MSC, NCC-MS #1, NCC-MS #2 and NCC-TX and 50% mortality to 6- to 12-leaf-stage plants of biotypes NCC-AR and NCC-IL when treated with the same rate and subjected to 8-h dew period prior to transferring to the greenhouse (Table 1).

The chemical herbicides chlorimuron, imazaquin, MSMA and imazethapyr were examined for herbicidal effects over a range of concentrations from a quarter to four times the manufacturer's recommended levels. Each herbicide was very active against MSC (Fig 1c and d), killing all or most

Table 1. Growth response of multiple seeded cocklebur verses various common cocklebur biotypes treated with *Alternaria helianthi* and herbicides

Response to treatment/biotype[a]	Treatment[b] (rate of application): Control	*A helianthi*	Chlorimuron (14.8 g ha^{-1})	Imazaquin (29.6 g ha^{-1})	MSMA (279.1 g ha^{-1})	Imazethapyr (39.5 g ha^{-1})
Plant height (cm)						
MSC-TX	21.1 (±2.6)[d]	0	0	0	0	0 ± 0
NCC-TX	20.3 (±1.9)[d]	0	0	0	0	0
NCC-AR	15.0 (±2.9)[c,d]	3.3 (±1.1)[e]	10.8 (±4.7)[e]	16.5 (±4.9)[e]	0[e]	16.0 (±2.2)[e]
NCC-IL	15.8 (±1.7)[c,d]	4.6 (±3.1)[e]	0.8 (±0.5)[e]	10.8 (±6.3)[e]	0[e]	5.8 (±4.3)[e]
NCC-MS#1	21.0 (±4.4)[d]	0	6.5 (±2.6)[e]	0[e]	0.4 (±0.5)[e]	12.8 (±4.6)[e]
NCC-MS#2	25.4 (±5.4)[d]	0	0[e]	0[e]	1.9 (±1.3)[e]	0
Dry weight (g)						
MSC-TX	1.9 (±0.5)[d]	0	0	0	0	0
NCC-TX	2.0 (±0.5)[d]	0	0	0	0	0
NCC-AR	1.3 (±0.4)[d]	0.5 (±0.1)[e]	1.1 (±0.4)[e]	1.6 (±0.5)[e]	0[e]	1.9 (±0.8)[e]
NCC-IL	1.8 (±0.4)[d]	0.8 (±0.3)[e]	1.2 (±0.6)[e]	1.0 (±0.3)[e]	0[e]	1.2 (±0.4)[e]
NCC-MS#1	2.4 (±0.4)[d]	0	2.0 (±0.3)[e]	0	0.2 (±0.3)	2.5 (±1.0)[e]
NCC-MS#2	3.1 (±0.9)[d]	0	0	0	0.6 (±0.7)	0
Mortality (%)						
MSC-TX	0	100	100	100	100	100
NCC-TX	0	100	100	100	100	100
NCC-AR	0	50 (±0)	0	0	100	0
NCC-IL	0	50 (±0)	0	0	100	0
NCC-MS#1	0	100	0	100	80 (±0)	0
NCC-MS#2	0	100	100	100	75 (±0)	100

[a] MSC-TX = multiple seeded cocklebur-Texas; NCC-TX = normal common cocklebur-Texas; NCC-AR = normal common cocklebur-Arkansas which is chlorimuron-resistant; NCC-IL = normal common cocklebur-Illinois which is imazethapyr-tolerant; and NCC-MS#1 & NCC-MS#2 = normal common cocklebur-Mississippi #1 & #2.

[b] Means were from two separate experiments, each consisting of four replications each containing 10 plants (one plant per pot) at 6- to 12-leaf-stage per replication. Results are the mean (± SD) 14 days after treatment.

[c] This biotype is significantly different from the other biotypes in height for the control and in resistance to chemical and fungal treatments as measured by height or weight (Exact Wilcoxon test, $P < 0.05$).

[d] This control was significantly different from all fungal and chemical treatments on the same biotype (Kruskal–Wallis test, $P < 0.02$). The chemical treatments on the biotype were not significantly different from each other (Exact Wilcoxon test, $P < 0.05$) unless indicated (see Note c).

[e] This chemical treatment was significantly more effective with the indicated biotype than chemical treatments not labeled with a 'c', but it was not significantly more effective than other chemical treatments of that biotype which are labeled with a 'c' (Exact Wilcoxon test, $P < 0.05$).

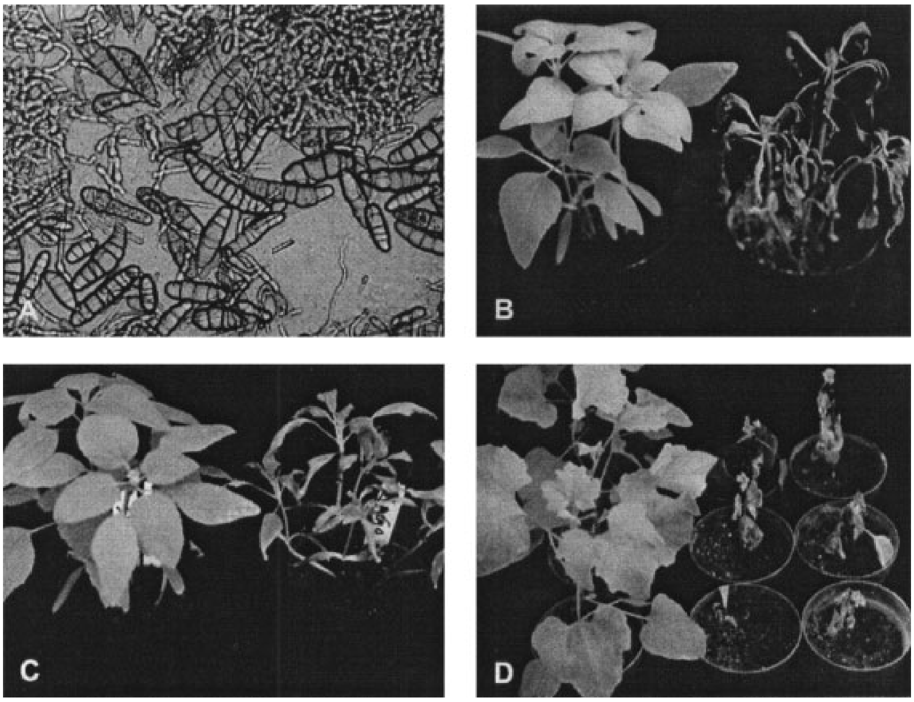

Figure 1. Effects of control agents on growth of multiple seeded cocklebur (MSC-TX). (A) Fungal conidia of *Alternaria helianthi* produced by fungus at 18 °C and used to treat common cocklebur plants. (B) MSC seedlings treated with 1×10^5 conidia ml^{-1} in 2 g $liter^{-1}$ aqueous Silwet L-77 on right and control plants on left (which received 2 g $liter^{-1}$ Silwet only). Note the disease symptoms on the treated plants (on right) which developed within 72 h. (C) MSC seedlings treated with MSMA at a rate of 279 g ha^{-1}. Note herbicidal damage on the treated plants on right which developed within one week. (D) Plants treated with chlorimuron at rates of 14.8 g ha^{-1} or 9.9 g ha^{-1} on MSC-TX (right), NCC-TX (middle), and NCC-AK (left). Note the same herbicidal damage occurred on two biotypes (right and middle) and herbicidal effect on NCC-AK at 118.5 g ha^{-1} which showed no damage because this biotype is resistant to chlorimuron.

when applied on top of 8- to 12-leaf-stage plants. Each of these chemical herbicides were effective (Kruskal–Wallis test on height and weight data, $P < 0.05$) with all multiple seeded cocklebur and most normal seeded cocklebur at application rates below the manufacturer's recommended treatment levels (chlorimuron: 6 g $acre^{-1}$; 14.8 g ha^{-1}, imazaquin: 12 g $acre^{-1}$; 29.6 g ha^{-1}, MSMA: 113 g $acre^{-1}$; 279.1 g ha^{-1} and imazethapyr: 16 g $acre^{-1}$; 39.5 g ha^{-1}; Table 1). For either multiple seeded or normal seeded cocklebur from Texas there was no significant difference between the effectiveness of any of the chemical treatments as used (Exact Wilcoxon test on height and weight data, $P < 0.05$). For normal seeded cocklebur from other places, MSMA was significantly more effective (Exact Wilcoxon test on height and weight data, $P < 0.05$) than other chemical treatments except with NCC-MS#2. The difference in effectiveness may reflect different mechanisms of action of the herbicides, because MSMA is a membrane disruptor of the organic arsenical class, whereas the other chemical herbicides are members of the more environmentally acceptable class of herbicides which inhibit branched-chain amino acid synthesis by acting on acetolactate synthase.[34] NCC-MS#1, but not NCC-MS#2, exhibited significantly greater resistance to imazethapyr than to imazaquin, a structurally related imidazolinone inhibitor of branched-chain amino acid synthesis. These sensitivity differences may reflect differences in prior herbicide exposure in the two NCC populations from Mississippi.

Among the five normal seeded cocklebur biotypes included in this study two, NCC-AR and NCC-IL, were significantly shorter than MSC-TX even in the control group without chemical or fungal treatment. However, the dry weights of these two biotypes were not significantly lower, indicating a 'bunchier' shape. In the present study this 'bunchiness' characteristic was significantly (Exact Wilcoxon test on height and weight data, $P < 0.05$) associated with resistance to *A helianthi* treatment and to treatment with chemical herbicides that inhibit branched-chain amino acid synthesis, but not with resistance to the arsenical herbicide MSMA. Among the branched-chain amino acid synthesis inhibitors, the two members of the imidazolinone structural class (imazaquin and imazethapyr) were less effective than chlorimuron, a member of the sulfonylurea structural class, particularly against the bunchy biotypes, NCC-AR and NCC-IL (Exact Wilcoxon test on height and weight data, $P < 0.05$). Further study will be needed to establish the generality of the association of the bunchiness characteristic with reduced herbicide effectiveness, and to identify any underlying mechanism(s).

The effectiveness of *A helianthi* against common cocklebur of all biotypes can be enhanced by the use of Silwet L-77 as adjuvant.[25] This will make biological control of common cocklebur more feasible and agrees with previous studies.[26,35] The precise mode by which such surface-active agents enhance conidial germination has yet to be determined. Some research has demonstrated that Silwet promotes the activity of bacteria and fungi on the leaves of their host kudzu (*Pueraria* sp).[35,36]

There have been concerns that, if multiple seeded cocklebur escapes control, it may spread more rapidly, because it produces several more seedlings per bur. However, the results of the current study indicate that MSC-TX is very susceptible to both biological and conventional chemical herbicide control methods. Therefore, no new strategies appear to be needed to control this novel biotype.

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